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Molecular Characterization of Begomovirus Infecting Ornamental Plants in Northern India

Synopsis

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INTRODUCTION

Ornamental plants are plants that are grown for decorative purposes in gardens and landscape design projects, as houseplants, for cut flowers and specimen display (Marwal *et al.*, 2013a). The cultivation of these forms a major branch of horticulture. Most commonly ornamental garden plants are grown for the display of aesthetic features including: flowers, leaves, scent, overall foliage texture, fruit, stem and bark, and aesthetic form (Marwal *et al.*, 2013b). In some cases, unusual features may be considered to be of interest, such as the prominent and rather vicious. In all cases, their purpose is for the enjoyment of gardeners, visitors, and/or the public. Similarly certain trees may be called ornamental trees. This term is used when they are used as part of a garden or landscape setting, for instance for their flowers, their texture, form and shape, and other aesthetic characteristics. The term ornamental plant is used here in the same sense that it is generally used in the horticultural trades. The term largely corresponds to 'garden plant', though the latter is much less precise, as any plant may be grown in a garden (Marwal *et al.*, 2013c).

Viruses are the smallest biological structures that have the ability to replicate only inside living cells. Essentially they consist of a shell of protein which encloses a core of nucleic acid – either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). There are over 700 known plant viruses, many of which cause catastrophic diseases and have wide host ranges. They have been classified into three families and 32 groups (Martelli, 1992). Serological and nucleic acid based techniques are being used for establish the identity or relatedness of plant viruses. Viruses with single stranded DNA that infect plants belong primarily to the family *Geminiviridae* and, a few of them, to the family *Circoviridae*.

Geminiviruses were recognized in 1978 by the International Committee on the Taxonomy of viruses on the basis of their unique virion morphology and possession of ssDNA as their genomic material (Matthews, 1979; Goodman, 1981). The family *Geminiviridae* is one of the largest plant virus family; its members have a circular, single-stranded DNA (ssDNA) genome of approximately 2.7–5.2 kb encapsulated within twinned (geminate) icosahedral virions. The protein coat of geminiviridae consists of one type protein molecule of about 28kd molecular weight. Based on their genome arrangement and biological properties, geminiviruses are classified into one of four genera: Mastrevirus, Curtovirus, Topocuvirus and Begomovirus (Stanley *et al.*, 2005). Whereas Mastreviruses and Curtoviruses are transmitted via various leafhoppers, Begomoviruses are transmitted by the whitefly (*Bemisia tabaci*) and Topocuvirus is transmitted by the treehopper. The construction of the genome of geminiviruses, regardless of the genus to which they belong, is quite complex. Because geminiviruses replicate by producing temporary complementary (-) ssDNA strand, both the original

virus (+) ssDNA and newly formed (-) ssDNA can have open reading frama (ORFs) that may be translated into functional proteins (>10kd). Transcription of mRNA and subsequent translation into protein can take place in both directions in each of the ssDNAs.

Bemisia tabaci commonly found in tropical and sub-tropical regions, and in greenhouses of temperate areas. The Northern India supports such type of climates for the breeding of whitefly. *B. tabaci* was first described in the genus Aleyrodes in 1889 (Gennadius, 1889), and was first reported as a pest in 1919 in India (Husain and Trehan, 1993). Since then, B. *tabaci* has been recognized as a pest of crops in tropical and subtropical countries. *B. tabaci* has a very wide host range, consisting of 500 species in 74 plant families (Greathead, 1986). The whitefly is a vector of viruses in the *Geminiviridae, Potyviridae* and *Comoviridae* families and the genera Carlavirus and Closterovirus.

With the development of reliable computational recombination detection tools and an increasing number of available genome sequences, many studies have reported evidence of recombination in a wide range of virus genera. The computational analysis suggested that interspecific recombination has resulted in remarkable diversity among geminiviruses and could be a major cause of the emergence of new geminivirus diseases (Marwal *et al.*, 2011). Ornamental plants are widely distributed in the India and have high environmental adaptability. Such ornamentals are considered as a source of new viruses and reservoirs of unidentified economically important viruses but are often neglected during diversity studies. Thus, there was a pressing need for additional information on the diversity and distribution of begomoviruses weeds, which likely serve as virus reservoirs.

OBJECTIVES

The precise objectives of this research work are as follows:

1. Survey for the Recognition of Begomovirus Symptoms in Ornamental Plants in Northern India.
2. Molecular Characterization of Begomoviral DNA-A, DNA-B, DNA-α and DNA-β Satellites in Ornamental plant species through PCR and Detection of virus Nucleic acid by Dot Blot Hybridization
3. Complete Genome Amplification by RCA of the Begomovirus and its Satellite molecules.
4. Construction of Genomic map, Phylogenetic and Nucleotide Sequence Analysis of identified Begomoviruses and other homologous and Report of new disease.
5. Comparative Homology Modeling, Prediction of binding sites in begomovirus protein and ligand finding studies of the identified begomovirus.
6. Recombination analysis and genome relationships study, contributing to the begomovirus evolution.

REVIEW OF LITERATURE

The largest genus, Begomovirus, currently contains 200 species (Fauquet *et al.*, 2008) that contains dicotyledonous infecting whitefly transmitted viruses in the family *Geminiviridae*, have either bipartite genomes (DNA-A and DNA-B) or monopartite genomes resembling DNA-A. DNA-A typically have six open reading frames (ORFs): AV1/V1 (coat protein, CP) and AV2/V2 (AV2/V2 protein) on the virion-sense strand, and AC1/C1 (replication initiation protein, Rep), AC2/C2 (transcriptional activator, TrAP), AC3/C3 (replication enhancer, REn) and AC4/C4 (AC4/C4 protein) on the complementary-sense strand. DNA-B has two ORFs, encoding movement proteins: BV1 (nuclear shuttle protein, NSP) on the virus-sense strand and BC1 (movement protein, MP) on the complementary-sense strand (Rojas *et al.*, 2005). Begomoviruses species those have bipartite genomes, comprising a DNA-A component required for replication and encapsidation and a DNA-B component required for virus movement (Hanley *et al.*, 2000). The International Committee on Taxonomy of Viruses database (ICTVdB) provide virus code to begomovirus, that is 00.029.0.03, virus accession number is 029003GE and NCBI Taxonomy ID is 10814 (Fauquet *et al.*, 2005).

The opposing transcription units of begomovirus DNA-A and DNA-B molecules are separated by an intergenic region (IR) that generally shares a highly conserved region of approximately 200 nt, named the common region (CR) (Lazarowitz, 1992). The CR contains an origin of replication (ori) that includes a stem–loop structure containing an invariant nonanucleotide (TAATATTAC) sequence whose T7–A8 site is required for cleaving and joining viral DNA during replication and conserved iterated sequences required for specific recognition and binding by Rep during replication (Arguello-Astorga *et al.*, 1994; Fontes *et al.*, 1994).

Based on phylogenetic studies and genome arrangement, begomoviruses have been divided broadly into two groups: the Old World (OW) viruses (Eastern hemisphere, Europe, Africa, Asia) and the New World (NW) viruses (western hemisphere, the Americas) (Paximadis *et al.*, 1999; Rybicki, 1994). Begomovirus genomes have a number of characteristics that distinguish Old World and New World viruses. All indigenous New World begomoviruses are bipartite, whereas both bipartite and monopartite begomoviruses are present in the Old World. In addition, DNA-A of bipartite begomoviruses from the New World lacks an AV2 ORF (Rybicki, 1994; Stanley *et al.*, 2005). New World begomoviruses also have an N-terminal PWRSMAGT motif in the CP that is absent from Old World viruses (Harrison *et al.*, 2002).

The DNA A of the bipartite species is similar in arrangement to the genome of the monopartite begomoviruses. For New World bipartite begomoviruses, the DNA component lacks the V2 gene. The DNA-B component encodes BV1 and BC1, proteins that are essential for cell to cell

and systemic movement (Noueiry *et al.*, 1994; Sanderfoot *et al.*, 1996), and can influence host range (Ingham *et al.*, 1995). Although not directly involved in interaction with the whitefly vector, DNA-B sequences affect the efficiency of virus acquisition by the insect by determining the location of begomoviruses in plant tissues (Liu *et al.*, 1997). Mixed infection with two or more geminiviruses are common in tropical and subtropical areas (Ribeiro *et al.*, 2003). Virus interactions during such mixed infections could play an important role leading to the development of complex diseases (Fondong *et al.*, 2000; Monci *et al.*, 2002).

The soil-borne plant pathogen *Agrobacterium tumefaciens* supported the replication of tomato leaf curl geminivirus (Australian isolate) (TLCV) DNA. Replicative DNA forms of three other geminiviruses also accumulated at varying levels in *Agrobacterium*. Geminiviral DNA constructs that lacked the ability to replicate in *Agrobacterium* were rendered replication-competent by changing their configuration so that two copies of the viral *ori* were present. The low-level replication of TLCV DNA can occur in *Escherichia coli* containing a dimeric TLCV construct in a high copy number plasmid (Selth *et al.*, 2002).*Ageratum conyzoides* exhibiting yellow vein symptoms, collected near Haikou, Hainan Province, China, contained begomoviral DNA-A like molecules. There is less specific interaction between monopartite begomovirus and DNAβ than between the DNA-A and DNA-B of begomoviruses with bipartite genomes (Xiong *et al.*, 2006; Ilyas *et al.*, 2013).

Approximately 60 different geminiviruses had been reported to be transmitted by *B. tabaci* (Markam *et al.*, 1994). In the New World before 1986, *B. tabaci* was considered a pest of a limited number of crops (tobacco, cotton, potato, bean, soybean), but by 1986 a sudden increase of the whitefly population in ornamentals in Florida was observed (Osborne, 1988). Shortly after that, whiteflies were reported in other crops in Florida, in California (Perring *et al.*, 1991), and in Texas, Arizona, Central America and South America (Brown, 1994). In Florida the whitefly infestation was associated with silver leaf of squash and irregular ripening of tomato (Maynard and Cantliffe, 1989).

METHODOLOGY

1. **Collection of plant samples:** The leaf samples were collected from the different regions of North India on the basis of typical begomovirus symptoms i.e., yellowing of leaf veins, yellow mosaics patterns, curling of fractional or complete leaves, leaf distortion, chlorosis and decrease in leaf size and stunting in plants.
2. **DNA extraction and PCR amplification:** Total DNA from leaf samples were extracted by using a CTAB (Cetyl Trimethyl Ammonium Bromide) method. The DNA pellet was washed with cold 70% ethanol twice, vacuum dried, and resuspended in 100 µl of TE buffer (Manen *et al.*, 2005). Concentration of DNA was estimated through NanoDrop (Cuvette free spectrophotometer) to check the quantity of DNA per microliter for further study. PCR was carried out in 15 µl volumes by using begomovirus specific universal primers against DNA A, DNA B, α and β components (Marwal *et al.*, 2013d). This confirmed the molecular level infection of the begomovirus in plant samples. After PCR amplification the gel electrophoresis technique was used for the separation of deoxyribonucleic acid (DNA). 1 % agarose was prepared in 1 ml TAE buffer with 2 µl Ethidium Bromide (EtBr) for making electrophoresis gel.
3. **Sequencing of DNA samples:** Sequencing of the amplified product was carried out by using ABI automated sequencer present at TCGA (The Centre for Genomic Application) New Delhi and Chromous Biotech Hyderabad.
4. **Submission of sequences in National Centre for Biotechnology Information (NCBI) and Homology search:** After sequencing the DNA sequence was submitted to NCBI-BankIt or Sequin. Homology search was carryout by using alignment tools (e.g. NCBI-BLASTn etc).
5. **Phylogenetic study:** Database search was carried out with BLAST*n* or with other BLAST tools. MEGA version 4.0 (http://www.megasoftware.net) were used for Pair wise and Multiple sequence alignments through its CLUSTAL- W program and Phylogenetic trees was constructed by using Neighbour-Joining algorithm.
6. **Recombination analysis using RDP and sequence analysis / validation:** Nucleotides sequence information was further analysed to find out Recombination in begomovirus isolate using Recombination Detection Program (RDP). Nucleotides sequences were further used to the refinement and validation of different patterns and energy errors in experimental models of CDS structures.

SUMMARY OF THE FINDINGS

Survey and Genome amplification

The present study was undertaken for complete genome amplification, molecular characterization, nucleotide sequence analysis and protein analysis of begomovirus and its components in different geographical regions of north India. During survey in four states of north India (Rajasthan, Haryana, New Delhi and Punjab), Marigold (*Tagetes patula*) and Rose (*Rose indica*) was found infected with begomovirus associated with their satellites. Preliminary identification of begomovirus and its satellite molecules was carried out by PCR and final confirmation was done by Dot Blot Hybridization. A PCR product of ~550 bp using the universal coat protein primer (AC-1048 f, AV-494 r) was obtained as a positive confirmation of begomovirus.

The amplification up to expected size ~2.7 kb and ~1.3 kb were obtained for the begomovirus isolates from Marigold (*Tagetes patula*) and Rose (*Rose indica*) using the TempliPhi-100 Amplification kit, after the restriction *digestion* of RCA products with *Eco*RI, *Bam*HI, *Sal*I and *HindIII* enzymes. Amplified RCA products up to expected size were cloned, sequenced and submitted to NCBI through BankIt and Sequin. The begomovirus infecting Marigold were identified as *Ageratum enation virus* (AEV: KC589699) and the begomovirus isolated from Rose was identified as *Rose leaf curl virus* (RoLCV: KF584008). DNA-β associated with Marigold and Rose were identified as *Ageratum leaf curl betasatellite* (ALCB: KC589700) and *Rose leaf curl betasatellite* (RoLCB: KF584009) respectively. Based on the nucleotide sequence BLAST analysis of the alphasatellite associated with Marigold, we propose the name *Marigold leaf curl alphasatellite* (MLCuA: KC206078).

The DNA-A of Marigold (*Tagetes patula*) and Rose (*Rose indica*) contains six predicted open reading frames (ORFs AV1, AV2, AC1, AC2, AC3 and AC4) that are conserved among begomoviral DNA-A. DNA-β associated with Marigold and consisted of an ORF C1 located on the complementary-sense strand encoding a protein of 142 and 108 amino acids. The complete nucleotide sequence of the alphasatellite isolated from Marigold was determined, having Rep gene in the sense strand.

Phylogenetic analysis

Phylogenetic analysis indicates that isolate M 2 cluster with the isolates of *Ageratum enation virus* from India. The DNA-A sequences of the M 2 isolate had high nucleotide sequence identity (93-97%) with other begomoviruses reported from India (JF728861, FN794201, JQ911767, FN543099, and JX436472), Pakistan (AM701770, AM261836, AM698011) and Nepal (AJ437618), which are all isolates of *Ageratum enation virus*. Using MatGAT, Marigold showed almost similar amount of

identity with other begomovirus genome retrieved from NCBI i.e. the range of highest and lowest nucleotide identity is quite less (92 - 97 %).

Ageratum leaf curl betasatellite (ALCB: KC589700) showed highest sequence identity ranging from 92 to 96% reported from India (JQ408218, JF728868, JQ710745, AM412239) and Pakistan (AM701771, AM698010) which are isolates of *Ageratum leaf curl betasatellite* and *Ageratum yellow leaf curl betasatellite*. MatGAT also showed the same results.

In phylogenetic comparisons the Marigold alphasatellite forms a cluster with various alphasatellites reported from Pakistan (AM884370, AM930244), AM930248, AM930245) and India (HE861941, JX512905, FN794199, AJ512959). It was most closely related to alphasatellite rep gene for truncated replication associated protein, clone UK7 [Pakistan: Faisalabad: 2010] (AM930246), with 93% nucleotide sequence identity. Global alignment revealed a range of 40 - 42 % nucleotide identity of Marigold alphasatellite with other NCBI isolates. Our discovery showed a begomovirus and its satellites were found associated with Marigold and the disease had not been investigated previously, therefore to our knowledge this is the first report of molecular characterization of marigold-infecting begomovirus and their associated satellites molecules from India.

The begomovirus *Rose leaf curl virus* (RoLCV: KF584008) revealed highest nucleotide sequence identities of 98 % with *Rose leaf curl virus* (GQ478342) which infecting an ornamental plant Chinese rose in Pakistan. MatGAT global alignment revealed that begomovirus infecting Rose showed almost dissimilar amount of identity with other begomovirus genome retrieved from NCBI i.e. the range of highest and lowest nucleotide identity is quite high (76 - 96 %).

Whereas the *Rose leaf curl betasatellite* (RoLCB: KF584009) had highest nucleotide sequence identities of 97 % with *Rose leaf curl betasatellite* (GQ478344) infecting Chinese rose in Pakistan. Global alignment revealed a range of 51 - 96 % nucleotide identities with other NCBI isolates. The possible association of a begomovirus with Rose had not been investigated previously. To the best of our knowledge, this is the first report worldwide of the begomovirus and its betasatellite associated with leaf curl disease in an ornamental plant Rose.

GC profile analysis

Three segmented boundaries were detected in *Ageratum leaf curl betasatellite* (ALCB: KC589700). There is an abrupt decrease of the density of GC profile at the first boundary below zero ranging from 250 to 350 bp. Second boundary has a poorest negative region (G + C) between 1100 to 1500 bp and the third boundary is observed at 2600 to 2700 bp. The overall GC content of the DNA-A sequence was calculated as 42.03 %. Whereas in the case of *Ageratum leaf curl betasatellite* (ALCB:

KC589700) only a single stretch of low GC profile was observed from 650 to 1150 bp. The overall GC content of the betasatellite sequence was calculated as 38.50 %. In case of *Marigold leaf curl alphasatellite* (MLCuA: KC206078) there is a sharp decrease in the GC profile below zero from position 350 to 800 bp. The overall GC content of the alphasatellite sequence was calculated as 39.20 %.

In *Rose leaf curl virus* (RoLCV: KF584008) the first boundary marked from 200 to 350 bp. Second boundary has an intense decrease in the negative region (G + C) ranging from 1100 to 1600 bp. The overall GC content of the begomovirus sequence isolated from Rose was calculated as 44.64. *Rose leaf curl betasatellite* (RoLCB: KF584009) has a very good GC profile. Though two segmented boundaries were found, one spans from 150 to 250 bp and the second boundary ranges from 1000 to 1200 bp. None of the boundaries below zero was encountered with a GC content of 36.39 %.

Restriction Map

Restriction map of all the sequences were drawn using NEBcutter server. The six main ORFs in *Ageratum enation virus* (AEV: KC589699) i.e. AV2, AV1, AC3, AC2, AC1 and AC4 are flanked by sites BsiHKAI and BisIMutI; BsrGI and BsmI; MboII and Ms1I; SspI and MboII; BsaJI and Pf1FI; Cac8I and Ms1I respectively that could be used to excise them from the complete begomovirus sequence. In *Ageratum leaf curl betasatellite* (ALCB: KC589700) the single ORF; C1 is flanked by sites PsiI and NdeI. In case of *Marigold leaf curl alphasatellite* (MLCuA: KC206078) the single ORF Rep is flanked by BarI and Hpy166II.

The six main ORFs in *Rose leaf curl virus* (RoLCV: KF584008) i.e. AV2, AV1, AC3, AC2, AC1 and AC4 are flanked by sites DraI and Bs1I; BfaI and ApoI; AlwI and EcoNI; PshAI and AcuI; MwoI and BsrGI respectively. In *Rose leaf curl betasatellite* (RoLCB: KF584009) the single ORF; C1 is flanked by sites PsiI and NdeI.

Homology Modeling

The alignment of Marigold AC1 protein by using BLASTp revealed the highest 84% identity with Chain A, Minimized Average Structure of the N-Terminal, DNA-Binding Domain of the Replication Initiation Protein from a Geminivirus (*Tomato Yellow Leaf Curl Virus*-Sardinia) (1L2M_A). Similarly Rose AC1 protein also revealed highest 76 % identity with the same protein (1L2M_A). Therefore 1L2M_A was found suitable for the homology modeling. The PROCHECK results shows that, Ramachandran plot of query Marigold AC1 protein and Rose AC1 protein had only 77.9 % and 77.7 % residues in core/favored region. Ramachandran plot of 1L2M_A had 86.5 %

residues in core region; therefore, all of these models could not be placed in good quality. The z-scores of AC1 Rep protein for both the viruses were found to be -5.08 and that of 1L2M_A was computed as -5.03.

During BLASTp analysis of query Marigold AC2 protein sequence against PDB revealed 43% identity with Chain A, Cryo-structure of the *Paracoccus Denitrificans* four-subunit Cytochrome C Oxidase in the completely oxidized state complexed with an antibody Fv fragment (1AR1_A). Ramachandran plot of query Marigold AC2 protein (Figure 4.46a) sequence displayed only 71.0 % residues in core/favored region and its homologous template 1AR1_A showed 84.6 % in core. Whereas in case of Rose AC2 protein Chain A, Crystal Structure of Nectin-Like Molecule-1 Protein Domain 1 of Homo sapiens (human) (1Z9M_A) showed highest identity of 30 %, which was subsequently downloaded from the server. Further Rose AC2 protein has 78.8 % residues in core/favored region and 1Z9M_A. The z-scores of Marigold AC2 protein were found to be -0.92 and that of 1AR1_A was computed as -4.71. In case of Rose AC2 protein, a z-score of -1.74 was encountered and its homologous showed -5.14 as z-score.

The alignment of Marigold AC3 protein revealed the highest 31% identity with Chain A, X-Ray Structure of Acetylcholine Binding Protein (Achbp) of *Lymnaea stagnalis* (great pond snail) (1I9B_A). Whereas in Rose AC3, the Chain A, Crystal Structure of Faicar Synthetase (Purp) from *Methanocaldococcus Jannaschii* complexed with Amppcp and Aicar (2R7K_A) showed highest identity of 32 %. Ramachandran plot of query Marigold and Rose AC3 protein had only 80.8 % and 81.2 % residues respectively in core/favored region. Whereas their homologous had 86.6 % and 92.3 % residues in core/favored region respectively, therefore 2R7K_A can be place in good quality. The z-scores of AC3 protein were found to be 0.24 and that of 1I9B_A was computed as -5.3. In case of Rose AC3 protein, a z-score of 0.39 was encountered and its homologous 2R7K_A showed -10.51 as z-score.

The alignment of Marigold and Rose AC4 protein revealed the highest 35 % identity with Chain A, Crystal Structure of Human Type-I N-Myristoyltransferase with bound Myristoyl-CoA (3IU1_A) and Chain A, Crystal Structure of Virulent Allele Rop5b Pseudokinase Domain of *Toxoplasma gondii* (3Q5Z_A) respectively. The PROCHECK results shows that, Ramachandran plot of query Marigold AC4 protein and Rose AC4 protein had only 74.7 % and 78.4 % residues in core/favored region respectively. Ramachandran plot of 3IU1_A had 90.3 % residues and 3Q5Z_A had 91.3 % residues in core/favored region. Ramachandran plot of 3IU1_A and 3Q5Z_A had residues over 90 % in core/favored region, therefore they can be placed in good quality. The z-scores of AC4

protein were found to be -2.02 and that of 3IU1_A was computed as -10.05. In case of Rose AC4 protein, a z-score of -0.86 was encountered and its homologous 3Q5Z_A showed -6.47 as z-score.

The alignment of Marigold AV1 protein by using BLASTp revealed the highest 32 % identity with Chain A, Crystal Structure of the N-terminal Domain of *Helicobacter pylori* Caga Protein (4G0H_A). Whereas Rose AV1 protein also revealed highest 43 % identity with Chain A, Crystal Structure of ATP Synthase Subunit Alpha (Tm1612) from *Thermotoga Maritima* (2R9V_A). The PROCHECK results shows that, Ramachandran plot of query AV1 protein had 78.6 % residues and Rose AV1 had only 75.0 % residues in core/favored region. Ramachandran plot of 4G0H_A and 2R9V_A had 96.3 % and 92.6 % residues in the most favored respectively, therefore, 4G0H_A and 2R9V_A is placed in good quality. The z-scores of AV1 protein for both the viruses were found to be -1.07 and -1.15 respectively and that of 4G0H_A and 2R9V_A was computed as -6.79 and -10.27 respectively.

The alignment of Marigold AV2 protein and Rose AV2 protein by using BLASTp revealed the highest 43 % and 36 % identity with Chain A, structure of Polyketide Synthase associated protein 5 from *Mycobacterium tuberculosis* (1Q9J_A). Ramachandran plot of query Marigold and Rose AV2 protein had 95.6 % and 86.1 % residues in core/favored region, therefore Marigold AV2 protein is placed in good quality. Ramachandran plot of 1Q9J_A had 81.8 % residues in core. The z-scores of Marigold AV2 protein were found to be -1.36, Rose AV2 protein gives -3.04 and that of 1Q9J_A was computed as -6.97.

The alignment of Marigold βC1 protein by using BLASTp revealed the highest 31 % identity with Chain C, Structure of Triatoma Virus (Trv) (3NAP_C) and Rose βC1 protein by showed the highest 32 % identity with Chain A, Crystal Structure of *Arabidopsis Thaliana* Double Bond Reductase (At5g16970)-Apo Form (2J3H_A). Marigold βC1 protein had 77.5 % residues in core/favored region and Rose βC1 protein showed 85.9 % residues in core/favored region. Both their homologous protein showed 87.4 % residues in core region. The z-scores of Marigold βC1 protein were found to be -1.73 and that of 3NAP_C was computed as -4.01. The z-scores of Rose βC1 protein were found to be -0.41 and that of 2J3H_A was found to be -7.95.

At last the alphasatellite Rep protein revealed the highest 38 % identity with Chain Y, structure of Rabgdp-Dissociation Inhibitor in Complex with Prenylated Ypt1 Gtpase (1UKV_Y) isolated from *Saccharomyces cerevisiae* (baker's yeast). Rep protein had 79.7 % residues in core/favored region and 1UKV_Y had 92.3 % residues in the most favored, therefore 1UKV_Y can be placed in good quality. The z-scores of Rep protein were found to be -2.09 and that of 1UKV_Y was computed as -11.3.

Prediction of Binding Site

During the analysis of AC1 protein of Marigold and Rose infecting begomovirus it was found that NAG (N-Acetyl-D-Glucosamine) was present at the binding site. Their AC2 proteins respectively had FAD (Flavin-Adenine Dinucleotide) and MG (Magnesium Ion) acting as a ligand molecule. ADP (Adenosine-5'-Diphosphate) and FRU (Fructose) molecules were the ligand molecules encountered at the binding site of AC3 protein of Marigold and Rose infecting begomovirus respectively. Further in case of AC4 proteins MDO (4-Methylidene-5-One Peptide Derived Chromophore) and PO4 (Phosphate Ion) respectively acts as a ligand molecule / heterogen, very much present in the vicinity of the protein.

Both the AV1 proteins had CA (Calcium Ion) present at the binding site. The ligand responsible at the binding site was of AV2 proteins were identified as HEA (HEME-A) and GOL (Glycerol). The beta C1 proteins had MG (Magnesium Ion) and ZN (Zinc Ion) on their binding affinity. Whereas in the case of rep protein of *Marigold leaf curl alphasatelite*; the heterogen molecule was revealed to be NI (Nickel (II) Ion).

Recombination Analysis

The RDP plot of *Ageratum enation virus* (AEV: KC589699) revealed six recombination break point positions, from 2665^{th} - 287^{th}, 388^{th} - 935^{th}, 938^{th} - 1042^{th}, 1042^{th} - 1200^{th}, 1237^{th} - 1366^{th}, 2074^{th} - 2210^{th} positions and having p-value as 3.281×10^{-11}, 5.787×10^{-24}, 1.335×10^{-12}, 1.660×10^{-08}, 2.380×10^{-03}, 1.829×10^{-07} respectively. The interesting findings were the evolution of *Ageratum enation virus* (AEV: KC589699) DNA-A sequence from begomovirus prevailing in India, as well as from other isolates existing in Pakistan and China. Perhaps this is the first report of recombination in begomovirus infecting Marigold, which would provide significant information for understanding the diversity and evolution of begomoviruses in India.

While analyzing the betasatellite molecule associated with leaf curl disease of Marigold plants, a total of four recombination events were detected ranging from 717^{th} - 783^{th}, 902^{th} - 965^{th}, 1085^{th} - 1122^{th}, 1076^{th} - 1195^{th} positions having p-values as 2.947×10^{-14}, 6.005×10^{-03}, 7.086×10^{-11}, 1.130×10^{-12} respectively. The interesting findings were the evolution of *Ageratum leaf curl betasatellite* (ALCB: KC589700) sequence from betasatellites prevailing in India, as well as from other isolates existing in Pakistan. Therefore this is the first report of recombination in betasatellite infecting Marigold.

Remarkably the recombination was detected in a small portion of the rep ORF of the aplhasatellite. The beginning breakpoint position was 936^{th} and ending breakpoint position was 974^{th}.

Approximate p-value for this region was 2.686 x 10^{-06}. These data provide evidence of the significant contribution of recombination to the genetic diversification of emerging alphasatellite population structures in India from neighboring countries like Pakistan and China. Therefore this is the first report of recombination in alphasatellite infecting Marigold.

The RDP plot of *Rose leaf curl virus* (RoLCV: KF584008) sequence conservation displayed three recombination break point positions from 216^{th} - 1448^{th}, 1777^{th} - 1796^{th}, 2188^{th} - 2589^{th} positions with p-value as 8.283 x 10^{-18}, 0.335, 4.408 x 10^{-03} respectively. The fascinating conclusion we have drawn the evolution of *Rose leaf curl virus* (RoLCV: KF584008) DNA-A sequence from begomovirus prevailing in India, as well as from other isolates existing in Thailand, specially Pakistan and China. Hence it is the first report of recombination in begomovirus infecting Rose, which would provide significant information for understanding the diversity and evolution of begomoviruses in India.

While analyzing the betasatellite molecule associated with leaf curl disease of Rose plants, a total of five recombination events were detected in the genome 6^{th} - 34^{th}, 990^{th} - 1243^{th}, 0.922, 1081^{th} - 1121^{th}, 1156^{th} - 1231^{th}, 1256^{th} - 1292^{th} positions having p-values as 9.670 x 10^{-02}, 3.061 x 10^{-07}, 7.614 x 10^{-16}, 4.060 x 10^{-10} respectively. The interesting findings were the evolution of *Rose leaf curl betasatellite* (ALCB: KF584009) sequence from betasatellites prevailing in India, as well as from other isolates existing in Pakistan. Therefore this is the first report of recombination in betasatellite infecting Rose.

In conclusion, we have established the existence of leaf curl disease of Marigold (*Tagetes patula*) and Rose (*Rose indica*) caused by association of begomovirus and its satellites. This study supported evidence that virus associated with this disease was identified through biological and molecular characters as well as bioinformatics analysis. The genetic diversity, nucleotide and protein sequence analysis as well as the evaluation of entropy power were viewed against the phylogenetic background which highlights the variable region where possibility of recombination increases.

Moreover this is the first evidence for the detection of recombination events in such viruses. These viruses can be replicated by other begomovirus species which increases the possibility of recombination and reassortment events. This could lead to evolution of new recombinant viruses or begomovirus complexes with different biological properties. It is also possible that exchange of genetic constituents could extend the virus host range thereby emergence of new diseases in cultivated crop plants and other ornamental plants.

One factor favoring the spread of begomoviruses among these plants is that many dicotyledonous species in India are hosts of whiteflies of the *B. tabaci* complex, which are the known or likely vectors of all the viruses.

SIGNIFICANCE OF WORK

1. Favourable climatic condition of Northern India supports the high population of whitefly (*Bemisia tabaci*). This study gives the information about the natural host plants and transmission vector in Northern states.
2. Several weeds and crops plants serve as the natural host of Begomovirus. Weeds show biological competition with the field crops and in several cases allows the transmission of Begomovirus from weeds to ornamental plants. This will increase host range of Begomovirus and affect the charming of ornamental plants.
3. Begomovirus genomics and proteomics database helpful in understanding the nature of virus and genetic diversity.
4. Information about role of Begomovirus genes in disease symptoms and disease development.
5. Information about phylogenetic and evolutionary relationship of Indian Begomovirus strains with other worldwide distributed stains.

FUTURE PROSPECTS

1. The increasing occurrence of begomoviruses in India demands efforts to study their diversity in order to anticipate and monitor outbreaks as well as to understand the evolutionary forces driving the emergence of novel begomoviruses in ornamental species previously unaffected by these pathogens.
2. Identification of possible further begomoviruses infection in the country in order to assess the contribution each makes to losses with a view to devising quarantine and control strategies.
3. Advanced molecular techniques such RNAi will be used for development of transgenic plant resistance to begomovirus.
4. This will form the basis of our future investigations. Results of these techniques effectively applied for disease management, crop protection and development of such strategies at state and national level in India.

REFERENCES

1. Arguello-Astorga, G.R., Guevara-Gonzalrz, R.G., Herrera-Estrella, L.R., and Rivera-Bustamante, R.F. (1994). Gemenivirus replication origine have a group specific organization of iterative elements: A model for replication. *Virology*, 203: 302-1000.
2. Brown, J. K. (1994). Current status of *Bemisia tabaci* as a plant and virus vector in agroecosystems worldwide. *FAO Plant Prot. Bull*, 41: 3-32.
3. Fauquet, C.M., Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M. and Zhou, X. (2008). Geminivirus strain demarcation and nomenclature. *Archives of Virology*, 153: 783–821.
4. Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U. and Ball, L.A. (2005). Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, pp. 1259.
5. Fondong, V.N., Pita, J.S., Rey, M.E.C., DeKochko, A., Beachy, R.N. and Fauquet, C.M. (2000). Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in Cameroon. *Journal of General Virology*, 81: 287-297.
6. Fontes, E.P., Eagle, P.A., Sipe, P.S., Luckow, V.A. and Hanley-Bowdoin, L. (1994). Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. *J Biol Chem*, 269: 8459–8465.
7. Gennadius, P. (1889). Disease of tobacco plantations in the Trikonia. The aleurodid of tobacco. *Ellenike Georgia*, 5:1-3.
8. Goodman, R. M. (1981). Geminiviruses: the single-stranded DNA viruses of plants. In Handbook of Plant Virus Infections. Amsterdam: North-Holland/Elsevier.
9. Greathead, A.H. (1986). Host plants. In Bemisia tabaci-A literature Survey. M. J. W. Cock (Ed.), CAB International Institute of Biological Control, Silwood Park, Ascot, Berks., UK, pp.17 - 25.
10. Hanley, B.L., Settlage, S.B., Orozco, B. M., Nagar, S. and Robertson, D. (2000). Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Biochemistry and Molecular Biology*, 35: 105-140.
11. Harrison, B.D., Swanson, M.M. and Fargette, D. (2002). Begomovirus coat protein: serology, variation and functions. *Physiological and Molecular Plant Pathology*, 60 (5): 257-271.
12. Husain, M.A. and Trehan, K.N. (1993). Observations on the life-history, bionomics and control of white-fly of cotton (*Bemisia gossypiperda* M. & L.). *Indian Journal of Agricultural Science*, 3:701 - 753.
13. Ilyas M, Nawaz K, Shafiq M, Haider MS, Shahid AA. (2013). Complete nucleotide sequences of two begomoviruses infecting Madagascar periwinkle (*Catharanthus roseus*) from Pakistan. *Archives of Virology*, 158: 505-510.

14. Ingham, D.J., Pascal, E. and Lazarowitz, S. G. (1995). Both bipartite geminivirus movement proteins define viral host range, but only BL1 determines viral pathogenicity. *Virology*, 207: 191-204.
15. Liu, S., Bedford, I. D., Briddon, R. W. and Markham, P. G. (1997). Efficient whitefly transmission of African cassava mosaic geminivirus requires sequences from both genomic components. *Journal of General Virology*, 78: 1791-1794.
16. Lazarowitz, S.G. (1992). Geminiviruses: genome structure and gene function. *Crit Rev Plant Sci*, 11: 327–349.
17. Malla, P., Roger, N.B. and Claude, M.F. (1995). Classification and identification of geminiviruses using sequence comparisons. *J Gen Virol*, 76: 249-263.
18. Matthews, R.E.F. (1979). Classification and nomenclature of viruses. *Intervirology*, 12: 129-296.
19. Markam, P. G., Bedford, S. L., and Pinner, M.S. (1994). The transmission of geminivirus by *Bemisia tabaci*. *Pestic. Sci*, 42: 123 - 128.
20. Martelli, G. P. (1992). Classification and nomenclature of plant viruses – state of the art. *Plant Disease*, 76: 436– 442.
21. Marwal A, Sahu AK, Gaur RK, (2013a). Evidence of the association of begomovirus and its betasatellite with the yellow vein disease of an ornamental plant *Calendula officinalis* (pot marigold) in Rajasthan, India: Molecular, Sequence and Recombination analysis. *Journal of Advances in Biology*, 1 (1): 29 - 44.
22. Marwal A, Sahu AK, Gaur RK, (2013b). First report of airborne begomovirus infection in *Melia azedarach* (Pride of India), an ornamental tree in India. *Aerobiologia*., DOI 10.1007/s10453-013-9319-x
23. Marwal A, Sahu AK, Gaur RK, (2013c). First report of molecular characterization of begomovirus infecting an ornamental plant *Tecoma stans* and a medicinal plant *Justicia adhatoda*. *Science International*. 25(4): 837-839.
24. Marwal A, Sahu AK, Gaur RK, (2013d). First report enlightening association of a plant pathogenic begomovirus with the leaf curl disease of an important ornamental crop *Citrus limon*: Can it act as a foodborne virus? *The Plant Pathology Journal. Photon.* 113: 150-155.
25. Marwal. A, Prajapat. R, Sahu. A, Gaur. R. K. (2011). In Silico Recombination analysis: A Study for Geminivurs Host Mobility. *Asian Journal of Biological Science*. [Accepted].].
26. Maynard, D.N. and Cantliffe D.J. (1989). Squash silver leaf and tomato irregular ripening: new vegetable disorders in Florida. University of Florida, IFAS, Florida Cooperative Extension Service, Vegetable Crops Fact Sheet VC-37.

27. Monci, F., Sanchez-Campos, S., Navas-Castillo, J. and Moriones, E. (2002). A natural recombinant between the geminiviruses Tomato yellow leaf curl Sardinia virus and Tomato yellow leaf curl virus exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. *Phytopathology*, 303: 317-326.

28. Noueiry, A.O., Lucas, W.J. and Gilbertson, R.L. (1994). Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport. *Cell*, 76: 925-932.

29. Osborne, L.S. (1988). The not so sweet sweetpotato whitefly. *Florida Foliage*, 14 (5):8-15.

30. Perring, T. M., Cooper, A.D., Rodriguez, R. J., Farrar, C. A. and Bellows, T.S. (1991). Identification of a whitefly species by genomic and behavioral studies. *Science*, 259: 74-77.

31. Paximadis, M., Idris, A.M., Torres-Jerez, I., Villarreal, A., Rey, M.E.C. and Brown, J.K. (1999). Characterization of tobacco geminiviruses in the Old and New World. *Arch Virol*, 144: 703–717.

32. Rybicki, E.P. (1994). A phylogenetic and evolutionary justification for 3 genera of Gemeniviridae. *Arch. Virol*, 139: 49-77.

33. Rojas, M.R., Hagen, W.J.L. and Gilbertson, R.L. (2005). Exploiting chicks in the plant armor. Evolution and emergence of gemeniviruses. *Annu. Rev. Phytopathol*, 43: 361-394.

34. Selth, L.A., John, W.R. and Rezaian, M.A. (2002). *Agrobacterium tumefaciens* supports DNA replication of diverse geminivirus types. *FEBS Letters*, 516 (1-3):179-182.

35. Stanley, J., Bisaro, D.M., Briddon, R.W., Brown, J.K., Fauquet, C.M., Harrison, B.D., Rybicki, E.P. and Stenger, D.C. (2005). Family Geminiviridae. In Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.

PUBLICATIONS

- Avinash Marwal, Anurag Kumar Sahu, Devendra Kumar Choudhary, R K Gaur (2013). Complete nucleotide sequence of a begomovirus associated with satellites molecules infecting a new host *Tagetes patula* in India. Virus Genes. 47: 194–198. **[Impact Factor = 1.3]**
- Avinash Marwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur (2013). First report of airborne begomovirus infection in *Melia azedarach* (Pride of India), an ornamental tree in India. Aerobiologia.. DOI 10.1007/s10453-013-931 **[Impact Factor = 1.3]**
- Rajarshi Kumar Gaur, Rajneesh Prajapat, Avinash Marwal, AnuragSahu, Mangal Singh Rathore (2011). First Report of a Begomovirus Infecting *Mimosa Pudica* in India. Journal of Plant Pathology. 93(4): S4.80. **[Impact Factor = 0.9]**
- Avinash Marwal, Anurag Sahu, Pradeep Sharma, Rajarshi Kumar Gaur (2013). Molecular Characterizations of Two Begomoviruses Infecting *Vinca rosea* and *Raphanus sativus* in India. Virologica sinica. 28 : 53 – 56. **[Impact Factor = 0.7]**
- Avinash Marwal, Anurag Sahu, Rajneesh Prajapat, Devendra Kumar Choudhary, Rajarshi Kumar Gaur (2012). First report of association of Begomovirus with the leaf curl disease of a common weed, *Datura inoxia*. Indian Journal of Virology. 23 (1): 83-84. **[Impact Factor = 0.3]**
- Avinash Marwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur (2013). Molecular characterization of a begomovirus infecting a new host Golden Duranta (*Duranta erecta*) in India. International Journal of Current Microbiology and Applied Sciences. 2 (9): 45 – 48. **[Impact Factor = 0.3]**
- Anurag Kumar Sahu, Avinash Marwal, Chita Nehra, Rajarshi Kumar Gaur (2013). Complete nucleotide sequence of a rose leaf curl disease and associated betasatellite infecting rose in India. Spanish Journal of Agricultural Research. [Communicated]. **[Impact Factor = 0.7]**
- Avinash Marwal, Anurag Sahu, Rajneesh Prajapat, Rajarshi Kumar Gaur (2013). First report of Begomovirus infecting two ornamental plants: *Jasminum sambac* and *Millingtonia hortensis*. Indian Phytopathology. 66 (1): 115 -116. **[NAAS Rating = 3.8]**
- Avinash Marwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur (2013). First report enlightening association of a plant pathogenic begomovirus with the leaf curl disease of an important ornamental crop *Citrus limon*: Can it act as a foodborne virus? The Journal of Plant Pathology. Photon. 113: 150-155. **[Impact Index = 3.7]**

- Avinash Marwal, Anurag Sahu, Rajneesh Prajapat, DK Choudhary, RK Gaur (2012). First Report of Begomovirus infecting two ornamental plants *Ocimum sanctum* and *Alternanthera variegata* in India. Journal of Clinical and Experimental Pathology. 2: 46. **[H Index = 16]**
- Rajneesh Prajapat, Avinash Marwal, Zuber Shaikh, Rajarshi Kumar Gaur (2012). Geminivirus Database (GVDB): First database of family Geminiviridae and its genera Begomovirus. Pakistan Journal of Biological Sciences. 15 (14): 702 – 706. **[H Index = 16]**
- Rajneesh Prajapat, Avinash Marwal, Rajarshi Kumar Gaur (2013). Begomovirus Associated with Alternative Host Weeds: A Critical Appraisal. Archives of Phytopathology and Plant Protection. DOI: 10.1080/03235408.2013.805497. **[H Index = 8]**
- Rajneesh Prajapat, Avinash Marwal, Anurag Sahu, RK Gaur (2012). Molecular In Silico structure and Recombination analysis of betasatellite in *Calotropis procera* associated with Begomovirus. Archives of Phytopathology and Plant Protection. 45: 1980 - 1990. **[H Index = 8]**
- Avinash Marwal, Anurag Sahu, RK Gaur (2013). First report of association of a begomovirus with *Chrysanthemum indicum*: An important ornamental plant exhibiting yellowing of leaf vein disease characterized by molecular studies. Journal of Horticultural Research. [Accepted].
- Avinash Marwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur (2013). Evidence of the association of begomovirus and an alphasatellite with the leaf curl disease of ornamental plant *Vinca alba* in Punjab, India. Journal of Agricultural Research. [Accepted].
- Avinash Marwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur (2013). Molecular characterization of begomoviruses and DNA satellites associated with a new host Spanish Flag (*Lantana camara*) in India. ISRN Virology. [Accepted].
- Avinash Marwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur (2013). First report of association of a begomovirus with Bougainvillea peruviana: An important ornamental plant exhibiting leaf curl disease characterized by molecular studies. Pakistan Journal of Phytopathology. [Communicated].
- Avinash Marwal, Anurag Sahu, RK Gaur (2013). Evidence of the association of begomovirus and its betasatellite with the yellow vein disease of an ornamental plant *Calendula officinalis* (pot marigold) in Rajasthan, India: Molecular, Sequence and Recombination analysis. Journal of Advances in Biology. 1: 29 - 44.
- Rajneesh Prajapat, Avinash Marwal, Rajarshi Kumar Gaur (2013). Recognition of errors in the Refinement and Validation of three-dimensional structures of AC1 proteins of Begomovirus strains by using ProSA-web. Journal of Viruses. [Accepted].

- Avinash Marwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur (2013). First report of molecular characterization of begomovirus infecting an ornamental plant Tecoma stans and a medicinal plant Justicia adhatoda. Science International. 25(4): 837-839.
- Avinash Marwal, Anurag Kumar Sahu, Rajneesh Prajapat, Devendra Kumar Choudhary, Rajarshi Kumar Gaur (2013). Molecular and Recombinational Characterization of Begomovirus infecting an ornamental plant Alternanthera sessilis: a new host of Tomato Leaf Curl Kerala Virus reported in India. Science International. 1 (3): 51 – 56.
- Rajneesh Prajapat, Avinash Marwal, Anurag Kumar Sahu, Rajarshi Kumar Gaur (2013). First Report of Begomovirus Infecting Sonchus asper in India. Science International. 1 (4): 108 – 110.
- Avinash Marwal, Rajneesh Prajapat, Anurag Sahu, Rajarshi Kumar Gaur (2012). Current Status of Geminivirus in India: RNAi Technology, A challenging cure. Asian Journal of Biological Science. 5 (6): 273-293.
- Avinash Marwal, Rajneesh Prajapat, Anurag Sahu, Rajarshi Kumar Gaur (2012). Computational characterization of Begomovirus infecting two ornamental plants: Jasminum sambac and Millingtonia hortensis. Asian Journal of Biological Science. 5 (5): 240-249.
- Avinash Marwal, Rajneesh Prajapat, Anurag Sahu, Rajarshi Kumar Gaur (2011). In Silico Recombination analysis: A Study for Geminivirus Host Mobility. Asian Journal of Biological Science. 5 (1): 1-8.
- Rajneesh Prajapat, Avinash Marwal, Anurag Sahu, Rajarshi Kumar Gaur (2011). Phylogenetics and in silico Docking Studies between Coat Protein of Mimosa Yellow Vein Virus and Whey α-lactalbumin. American Journal of Biochemistry and Molecular Biology. 1 (3):265-274.
- Rajneesh Prajapat, Avinash Marwal, Vivek Bajpai, Rajarshi Kumar Gaur (2011). Genomics and Proteomoics Characterization of Alphasatellite in Weed associated with Begomovirus. International Journal of Plant Pathology. 2 (1): 1-14.
- Rajneesh Prajapat, Rajarshi Kumar Gaur, Avinash Marwal (2011). Homology Modeling and Docking Studies between AC1 Rep Protein of Begomovirus and Whey α-lactalbumin. Asian Journal of Biological Sciences. 4 (4): 352-361.
- Avinash Marwal, Anurag Sahu, Pradeep Sharma, Rajarshi Kumar Gaur (2013). Transmission and Host Interaction of Geminivirus in Weeds. Plant Virus-Host Interaction: Molecular Approaches and Viral Evolution. Elsevier. Editors: Rajarshi Kumar Gaur, Thomas Hohn and Pradeep Sharma (In Press).

- Avinash Marwal, Anurag Sahu, Rajarshi Kumar Gaur (2013). RNA Silencing Suppressor Encoded by Betasatellite DNA Associated with Calendula officinalis yellow vein Lakshmangarh virus. "12th International Plant Virus Epidemiology (IPVE) Symposium, themed Evolution, Ecology and Control of Plant Viruses, Arusha, Tanzania.
- Anurag Kumar Sahu, Avinash Marwal, Pooja Sharma, R K Gaur (2012). "Assessment of Genetic Diversity within CP gene of Tomato leaf curl virus (ToLCV) infecting tomato plant across world". International Conference on Advances in Plant Science, Chian Mai, Thailand.
- Avinash Marwal, Anurag Sahu, Rajneesh Prajapat, Rajarshi Kumar Gaur (2012). Distribution and genetic diversity of Begomoviruses associated with ornamentals plants in India. "International Conference on Microbial, Plant & Animal Research" at Department of Science, Faculty of Arts, Science and Commerce, MITS University, India.
- Rajneesh Prajapat, Avinash Marwal, Anurag Sahu, Zuber Shaikh, Rajarshi Kumar Gaur (2012). Geminivirus Database (GVDB): Foremost approach of its kind. "International Conference on Microbial, Plant & Animal Research" at Department of Science, Faculty of Arts, Science and Commerce, MITS University, India.
- Rajneesh Prajapat, Avinash Marwal, Rajarshi Kumar Gaur (2011). The application of rolling circle amplification (RCA) for diagnosis and characterization of weed and crop associated Begomovirus. National conference on Development of new technologies in Biosciences, Gyan Vihar University, Jaipur, Rajasthan.
- Rajneesh Prajapat, Avinash Marwal, Anurag Sahu, Rajarshi Kumar Gaur (2011). "In Silico Characterization of Tomato leaf curl Joydebpur virus (ToLCJV) DNA-A Proteins".National Symposium on "Advances in Biotechnological Research in Agri-Horticultural Crops for Sustaining Productivity, Quality Improvement and Food Security", Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut (UP).
- Somyaparna Das, Avinash Marwal, Devendra Kumar Choudhary, Vijay Kumar Gupta, Rajarshi Kumar Gaur (2011). Mechanism of RNA interference (RNAi): Current concept. International Proceedings of Chemical, Biological and Environmental Engineering, 9: 240-245.
- Rajneesh Prajapat, Avinash Marwal, Rajarshi Kumar Gaur (2010). "Structural Modeling and Validation of Rep Proteins of Begomovirus Strains (TLCBV and TYLCTHV)". National Conference on Recent Advances in Integrated Disease Management for Enhancing Food Production. Organized By Department of Plant Pathology, College of Agriculture, SK Rajasthan Agricultural University, Bikaner, and Indian Phytopathological Society (Central Zone).